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(54) **MIRNA-124 AS A BIOMARKER**
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Description

[0001] The instant disclosure relates to the field of biomarkers, in particular in connection with viral infections.

[0002] More particularly, the invention relates to a novel biomarker useful as a diagnostic marker for viral infections.

The viral infections more particularly considered are viral infections requiring RNA splicing, and in particular retroviral infections such as HIV and AIDS-related conditions. The invention also relates to a follow-up marker for treatments of said infections, and in particular HIV and AIDS-related conditions.

[0003] In higher eukaryotes, messenger RNAs are not directly transcribed in their functional form but as pre-messenger RNAs which have to go through many processing events in order to be readable by the cellular translation machinery.

Splicing is the process which allows to eliminate the unwanted sequences (introns) and to join the meaningful ones (exons). The highly coordinated splicing event takes place in a large complex called the Spliceosome. The formation of this functional megacomplex is an orchestrated assembly of proteins and RNA that requires identification of exon-intron boundaries. Exons are regularly alternatively spliced, meaning that they are either included or excluded from the final mature mRNA transcript. A recent comprehensive sequencing study observed that more than 90% of the genes undergo alternative splicing. The production of alternatively spliced mRNAs is regulated by a system of trans-acting proteins that bind to cis-acting sites on the pre-mRNA itself. Such proteins include splicing activators that promote the usage of a particular splice site, and splicing repressors that reduce the usage of a particular site, binding on splicing enhancer sites (intronic splicing enhancers, ISE and exonic splicing enhancers, ESE) and on splicing silencer sites (intronic splicing silencers, ISS and exonic splicing silencers, ESS) respectively.

[0004] Viruses, in particular from the retroviral family, are one of the major causes of diseases around the world. Three subfamilies can be distinguished within the retroviral family: the oncoviruses, the lentiviruses and the spumaviruses.

[0005] The oncoviruses are thus termed because they can be associated with cancers and malignant infections. There may be mentioned, for example, leukemogenic viruses (such as the avian leukemia virus (ALV), the murine leukemia virus (MLV), also called Moloney virus, the feline leukemia virus (FELV), human leukemia viruses such as HTLV1 and HTLV2, the simian leukemia virus or STLV, the bovine leukemia virus or BLV, the primate type D oncoviruses, the type B oncoviruses which are inducers of mammary tumors, or oncoviruses which cause a rapid cancer (such as the Rous sarcoma virus or RSV).

[0006] The spumaviruses manifest fairly low specificity for a given cell type or a given species, and they are sometimes associated with immunosuppressive phenomena; that is the case, for example, for the simian foamy virus (or SFV).

[0007] The lentiviruses, such as HIV, are thus named because they are responsible for slow-progressing pathological conditions which very frequently involve immunosuppressive phenomena, including AIDS.

[0008] Viruses, and in particular retroviruses such as HIV, are known to rely upon RNA splicing and splicing regulation in order to spread and disseminate within cells and tissues of an infected individual.

[0009] Recently, the fact that HIV is a retrovirus that requires RNA splicing to express key viral proteins has been exploited to develop a novel strategy based on splicing inhibition to combat viral infections, and in particular AIDS (WO 2010/143169). Indeed, the HIV-1 genome expresses a primary transcript of 9 kb that not only serves as a genomic RNA for progeny virus, but which also generates 40 different mRNAs. HIV-1 uses four multiple alternative 5' splice sites and eight multiple alternative 3' splice sites to generate spliced mRNA species. These spliced mRNAs can be divided into two classes: multiply spliced (2 kb) and singly spliced (4 kb) RNAs. Regulation of HIV-1 alternative splicing occurs primarily because of the presence of suboptimal splicing sites which decrease the recognition by the cellular splicing machinery of the splice signals. Splicing at the viral splice sites is further regulated by the presence of ESEs, ESSs and ISSs.

[0010] In this context, quinoline derivatives have been developed, in particular 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine, which has been shown to inhibit replication in Peripheral Blood Mononuclear Cells (PBMC) of HIV-1 and HIV-2 T cell-tropic laboratory strains as well as clinical isolates of different subtypes at nM concentrations range (WO 2010/143169).

[0011] microRNAs (miRNA), the most comprehensive noncoding group, are a class of about 22 nt noncoding RNAs that inhibit gene expression through binding to the UnTranslated Region (UTR) of target mRNA transcripts (Lai et al., Nature Genetics, vol. 30, no. 4, pp. 363-364, 2002; Bartel et al., Cell, vol. 136, no. 2, pp. 215-233, 2009). miRNA genes represent about 1-2% of the known eukaryotic genomes. Predictions suggest that each miRNA can target more than 200 transcripts and that a single mRNA can be regulated by multiple miRNAs (LINDOW, DNA Cell Biol., vol. 26(5), p. 339-351, 2007). miRNAs are generated from endogenous hairpin-shaped transcripts and act by base pairing with target mRNAs, which leads to mRNA cleavage or translational repression, depending on the degree of base-pairing. Two processing events lead to mature miRNA formation: first, the nascent miRNA transcripts (pri-miRNA) are processed into 70 nucleotides precursors (pre-miRNA) which are exported from the nucleus and are cleaved in the cytoplasm to generate short (about 22 nucleotides long) mature miRNAs (LEE, EMBO J., vol. 21, p. 4663-4670, 2002). miRNAs can be located inter- or intragenically. When intergenic, their expression is coordinated with other miRNAs as a cluster (Altuvia et al., Nucleic Acids Research, vol. 33, no. 8, pp. 2697-2706, 2005; Oszolac et al., Genes and Development, vol. 22, no. 22,

pp. 3172-3183, 2008). When intragenic, namely, positioned within a protein-coding gene (almost exclusively in introns), they are often expressed from the same strand as their host-gene (Liu et al., Cell Research, vol. 18, no. 10, pp. 985-996, 2008, Kim et al., EMBO Journal, vol. 26, no. 3, pp. 775-783, 2007) and at correlated levels (Baskerville et al., RNA, vol. 11, no. 3, pp. 241-247, 2005).

[0012] miRNAs have recently been implicated in the intricate cross-talk between the host and the pathogen in viral infections and is thought to play a major role in viral pathogenesis (NAIR, Trends in Microbiol., vol. 14, p. 169-175, 2006). Indeed, viruses are obligate intracellular parasites using the cellular machinery for their survival and replication, so this dependence makes them susceptible to the host gene-regulatory mechanisms. Cellular miRNA can take part in an antiviral defence mechanism, but can, in some cases, also be viral positive regulators. On the other hand, viruses themselves can also produce miRNAs to regulate cellular processes or viral genes. miRNAs involved in HIV-1 infection could be defined as HIV-1-encoded or host-encoded according to their source of biogenesis; they could also be defined as suppressors or activators of infection according to their function. They can be further divided according to whether they directly target HIV-1 transcripts or indirectly affect HIV-1 by targeting host factors that are involved in virus life cycle, or targeting both the HIV-1 RNA genome and host factors essential for HIV-1 infection. Several data attest that HIV-1 infection affects miRNA pathways globally due to miRNA biogenesis perturbation but also individually by miRNA expression profiles modification (Houzet et al., Biochim Biophys Acta. 2011 Nov-Dec; 1809(11-12): 686-693)). Furthermore, host miRNAs have been described to regulate HIV-1.

[0013] One key factor for the success of development of a given drug or vaccine is the possibility to assess efficiently and rapidly its efficacy. Indeed, it is important for a given drug or vaccine to be administered in its therapeutic window so as to avoid unwanted effects coming from a too high dosage or to avoid a lack of efficiency due to a too low dosage. Also, one has to be sure that the proper drug or vaccine is administered to the proper patient, and that this patient is indeed responsive to the drug or vaccine. Therefore, simply linking together a given patient and a given drug or vaccine is not always enough to obtain a beneficial therapeutic effect. It is therefore critical to have proper tools, such as specific biomarkers, to rely upon for assessing the efficacy of a drug or vaccine.

[0014] Therefore, there is a need for a novel and sensitive tool for assessing a viral infection, and in particular a retroviral infection, and more particularly an HIV (Human Immunodeficiency Virus) infection, as well as the efficacy of a treatment of such conditions.

[0015] There is need for a novel biomarker for assessing the efficacy of a treatment of a viral infection, and in particular a retroviral infection, and more particularly an HIV infection.

[0016] There is a need for a novel and sensitive tool for assessing the efficiency of quinoline derivatives which are inhibitors of viruses, in particular retroviruses such as HIV, and more particularly HIV-1 and HIV-2.

[0017] There is a need for a novel biomarker for assessing the responsiveness of a patient to quinoline derivatives for preventing or treating a viral infection, and in particular a retroviral infection, and more particularly an HIV infection.

[0018] There is a need for a novel biomarker for assessing the therapeutic efficacy of quinoline derivatives for preventing or treating a viral infection, and in particular a retroviral infection, and more particularly an HIV infection.

[0019] There is also a need for a novel biomarker for screening drug candidates or vaccine effective for preventing and/or treating a viral infection, and in particular a retroviral infection, and more particularly an HIV infection.

[0020] The present invention has for purpose to meet these needs.

[0021] According to one of its objects, the invention concerns an *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker of a viral infection, or of an efficacy of a therapeutic treatment of said viral infection.

[0022] Unexpectedly, the inventors have observed, as detailed in the examples below, that, in PBMCs infected with an HIV strain, in particular with an ADA-M R5 HIV strain, the level of expression of miR-124 was decreased relative to non-infected PBMCs.

[0023] What is more, the inventors have unexpectedly observed that a treatment with quinoline derivatives, such as quinoline derivatives of formula (I) or (II), and in particular with the 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine, of peripheral blood mononuclear cells (PBMCs) infected with a HIV strain, in particular with the ADA-M R5 HIV, resulted in the removal of the viruses and in a dramatic increase (13-fold relative to control) of miR-124 expression. Quinoline derivatives may be chosen among the compounds described in WO 2010/143169 and as further described below. Accordingly, a therapeutic treatment of said viral infection can be a treatment with quinoline derivatives.

[0024] Accordingly, the miR-124 revealed itself as power tool, otherwise said as a biomarker, for monitoring a viral infection, in particular a retroviral infection, such as an HIV infection, in particular into individuals suffering from such an infection, as well as for monitoring individuals infected with a virus, in particular a retrovirus, such as an infection with HIV, and for monitoring such individuals treated with an antiviral drug, in particular with quinoline derivatives of formula (I) or (II) as detailed below, and in particular with 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine.

[0025] Thus, by monitoring the level of expression of miR-124, it is possible to track or perform quality control on human research trials or to monitor the patient compliance to a drug regimen or vaccine by providing a means to confirm that the patient is receiving appropriate drug or vaccine treatments, i.e., in terms of the dose and time. The miR-124

biomarker can also be used to optimize dosing regimens. Thus, miR-124 biomarker can be used in connection with, for example, the management of patient treatment, clinical trials, and cell-based research.

[0026] According to another of its objects, the invention concerns an *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker of a viral infection, preferably with a retrovirus, and more preferably with a Human Immunodeficiency Virus (HIV).

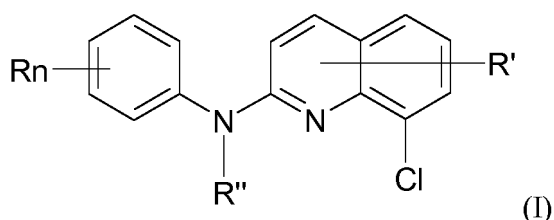
[0027] According to another of its objects, the invention pertains to an *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker for screening a drug candidate or vaccine candidate presumed effective in preventing and/or treating a viral infection, in particular a retroviral infection, and more particularly an HIV infection.

[0028] In particular, the efficacy of a therapeutic treatment of said viral infection is determined by assessing the biological effect of a candidate compound, in particular the pharmacological potential, of a candidate compound, to alter the physiological activity of a cell or a protein.

[0029] In this respect, it has been shown herein that the level of expression of miR-124 varies upon administration of various compounds that are known to possess a pharmacological activity. Thus, the inventors have shown that miR-124 consists of a relevant biomarker of the potential pharmacological activity of a candidate compound.

[0030] In particular, the drug candidate or vaccine candidate presumed effective in preventing and/or treating a viral infection can be a quinoline derivative.

[0031] In particular, the drug candidate or vaccine candidate presumed effective in preventing and/or treating a viral infection can be a quinoline derivative of formula (I):

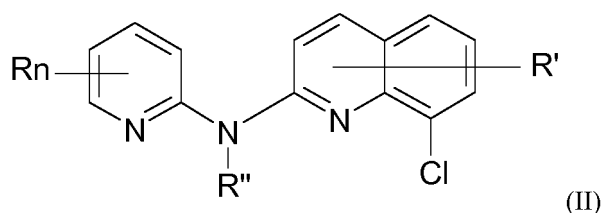


wherein

- n is 1 or 2 and R, independently, represents a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₃) alkyl group; a -NR₁R₂ group in which R₁ and R₂ are independently a hydrogen atom or a (C₁-C₃)alkyl group; a (C₁-C₃) fluoroalkoxy group; a -NO₂ group; a phenoxy group; and a (C₁-C₄) alkoxy group,
- R' is a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₄) alkyl group and a (C₁-C₄) alkoxy group,
- R'' is a hydrogen atom or a (C₁-C₄) alkyl group,

or one of its pharmaceutically acceptable salt.

[0032] The drug candidate or vaccine candidate presumed effective in preventing and/or treating a viral infection can also be a quinoline derivative of formula (II):



wherein:

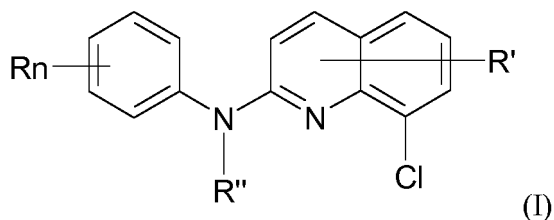
- n is 1 or 2 and R, independently, represents a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₃) alkyl group; a -CN group; a hydroxyl group; a -COOR₁ group; a (C₁-C₃)fluoroalkyl group; a -NO₂ group; a -NR₁R₂ group with R₁ and R₂ being a hydrogen atom or a (C₁-C₃)alkyl group; and a (C₁-C₄) alkoxy group,
- R' is a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₄) alkyl group and a (C₁-C₄) alkoxy group,
- R'' is a hydrogen atom or a (C₁-C₄) alkyl group,

or one of its pharmaceutically acceptable salt.

[0033] Within the invention, the term "preventing" intends to mean reducing the likelihood of occurrence of a given event, namely, in the context of the invention, a viral infection.

[0034] According to another of its objects, the invention concerns an *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker of an activity of a quinoline derivative, or one of its pharmaceutically acceptable salt, on a viral infection, in particular a retroviral infection, and more particularly an HIV infection.

[0035] According to another of its objects, the invention concerns an *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker of an activity of a quinoline derivative of formula (I):



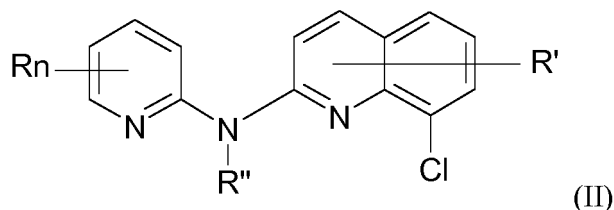
wherein

- n is 1 or 2 and R, independently, represents a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₃) alkyl group; a -NR₁R₂ group in which R₁ and R₂ are independently a hydrogen atom or a (C₁-C₃)alkyl group; a (C₁-C₃) fluoroalkoxy group; a -NO₂ group; a phenoxy group; and a (C₁-C₄) alkoxy group,
- R' is a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₄) alkyl group and a (C₁-C₄) alkoxy group,
- R'' is a hydrogen atom or a (C₁-C₄) alkyl group,

or one of its pharmaceutically acceptable salt,

on a viral infection, and in particular a retroviral infection, and more particularly an HIV infection.

[0036] According to another of its objects, the invention concerns an *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker of an activity of a quinoline derivative of formula (II):



wherein

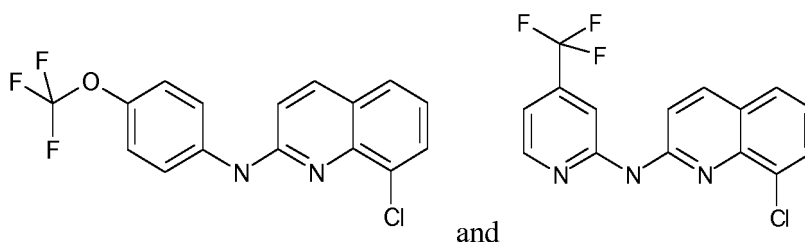
- n is 1 or 2 and R, independently, represents a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₃)alkyl group; a -CN group; a hydroxyl group; a -COOR₁ group; a (C₁-C₃)fluoroalkyl group; a -NO₂ group; a -NR₁R₂ group with R₁ and R₂ being a hydrogen atom or a (C₁-C₃)alkyl group; and a (C₁-C₄) alkoxy group,
- R' is a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₄) alkyl group and a (C₁-C₄) alkoxy group,
- R'' is a hydrogen atom or a (C₁-C₄) alkyl group,

or one of its pharmaceutically acceptable salt,

on a viral infection, in particular a retroviral infection, and more particularly an HIV infection.

[0037] According to a particular embodiment, a quinoline derivative may be 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine or 8-chloro-N-[4-(trifluoromethyl)pyridin-2-yl]quinolin-2-amine.

[0038] Thus, according to another of its objects, the invention concerns an *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker of an activity of a quinoline derivative selected from:



10 **[0039]** Within the invention, the expressions "viral infection" and "infection with a virus" refer to any viral infection, and in particular to any retroviral infection, which may occur into a cell, a tissue, an organ or an individual liable to express a biomarker of the invention. Preferably, a retroviral viral infection may be a lentiviral infection, and more preferably an HIV infection. An individual within the invention may be a mammal, and preferably a human liable to express a biomarker of the invention. Within the invention, individual and patient are used interchangeably.

15 **[0040]** Within the invention, the term "virus" refers to any virus, in particular a retrovirus and preferably a lentivirus such as an HIV virus, more preferably HIV-1 or HIV-2.

[0041] According to another of its objects, the invention pertains to an *in vitro* or *ex vivo* method for assessing a viral infection, and in particular a retroviral infection, and more particularly an HIV infection, in a patient presumed to be infected with a virus, comprising at least the steps of:

- 20
- a- measuring a presence or an expression level of at least one miRNA, said at least one miRNA being miR-124, in a biological sample previously obtained from said patient; and
 - b- comparing said presence or expression level to a control reference value,

25 wherein a modulated presence or level of expression of said miRNA relative to said control reference value is indicative of a viral infection.

[0042] According to another of its objects, the invention pertains to an *in vitro* or *ex vivo* method of assessing an activity of a quinoline derivative of formula (I) for preventing and/or treating a viral infection, in particular a retroviral infection, and more particularly an HIV infection, in a patient treated with said quinoline derivative, comprising at least the steps of:

- 30
- a- measuring a presence or an expression level of at least one miRNA, said at least one miRNA being miR-124, in a first biological sample previously obtained from said patient before administering said quinoline derivative and in a second biological sample previously obtained from said patient after administering said quinoline derivative; and
 - b- determining if said presence or expression level is modulated in the second biological sample obtained after the
- 35 treatment as compared to the second biological sample obtained before the treatment;

wherein a modulated presence or level of expression of said miRNA is indicative of an activity of said quinoline derivative.

[0043] "Biological sample," as used herein, generally refers to a sample obtained from a biological subject, including sample of biological tissue or fluid origin, obtained, reached, or collected *in vivo* or *in situ*. Such samples can be, but are not limited to, organs, tissues, fractions and cells isolated from a mammal. Exemplary biological samples include but are not limited to cell lysate, a cell culture, a cell line, a tissue, oral tissue, gastrointestinal tissue, an organ, an organelle, a biological fluid, a blood sample, a serum sample, a urine sample, a skin sample, and the like. Preferred biological samples include but are not limited to a blood, a plasma, a serum, a PBMC, a tissue biopsy, an oral mucosa, a saliva, an interstitial fluid, or an urine sample, and the like.

45 **[0044]** In one embodiment, a biological sample suitable for the invention may be selected in a group consisting of a biological tissue sample, a whole blood sample, a swab sample, a plasma sample, a serum sample, a saliva sample, a vaginal fluid sample, a sperm sample, a pharyngeal fluid sample, a bronchial fluid sample, a fecal fluid sample, a cerebrospinal fluid sample, a lacrymal fluid sample and a tissue culture supernatant sample.

[0045] The disclosure further relates to an isolated biological sample comprising a biomarker, wherein said biological sample is selected in a group comprising, and preferably consisting in a tissue sample, whole blood, swab sample, plasma, serum, saliva, vaginal fluid, sperm, pharyngeal fluid, bronchial fluid, fecal fluid, cerebrospinal fluid, lacrymal fluid and tissue culture supernatant; wherein said biomarker is a miRNA biomarker, and preferably miR-124.

50 **[0046]** According to another of its objects, the invention concerns an *in vitro* or *ex vivo* method for assessing the biological effect of a candidate compound and in particular for screening a drug candidate or vaccine candidate, presumed effective in preventing and/or treating a viral infection, and in particular a retroviral infection, and more particularly an HIV infection, comprising at least the steps of:

- 55
- a- treating at least one isolated cell able to express at least one miRNA, said at least one miRNA being miR-124,

with said candidate, said cell being under conditions suitable for expressing said at least one miRNA,
 b- measuring a presence or expression level of said at least one miRNA,
 c- comparing said measured presence or expression level with a measure or expression level of said at least one
 miRNA in an untreated isolated cell,

wherein a modulated presence or level of expression of said miRNA is indicative of a biological effect of a candidate compound and in particular of the efficacy of said drug candidate or vaccine candidate on a viral infection.

[0047] Within the invention, the terms "modulation" or "modulated presence or level of expression" intend to mean that the presence or level of expression of a biomarker of the invention is either induced or increased, or alternatively is suppressed or decreased.

[0048] Thus, it flows from the experimental results contained herein that miR-124, and notably the expression level of miR-124, consists of a relevant biomarker that is indicative of a physiological change of a protein or a cell, including a metabolic change of a cell, which change materializes a beneficial pharmacological effect.

[0049] Then, as stated previously, the disclosure also concerns the *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miRNA-124, for assessing the biological effect, in particular the pharmacological effect, of a candidate compound.

[0050] This disclosure also relates to the *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miRNA-124, for assessing the ability of a candidate compound to alter the physiological activity of a protein or a cell.

[0051] The alteration of the physiological activity of a protein or a cell may be easily determined by the one skilled in the art by identification of any detectable change in the measure of a physiological parameter of a cell, including in the measure of a metabolic parameter of cell, which encompasses electrophysiological changes, cell membrane permeability changes, enzyme activity changes, protein expression changes, miRNA expression changes, gene expression changes, intracellular pH values, etc.

[0052] The terms "determining," "measuring," "evaluating," "assessing," and "assaying," as used herein, generally refer to any form of measurement, and include determining if an element is present or not. These terms include both quantitative and/or qualitative determinations. Assessing may be relative or absolute. The phrase "assessing the presence of" can include determining the amount of something present, as well as determining whether it is present or absent.

[0053] According to one preferred embodiment, when assessing a viral infection, an observation of a reduced or suppressed presence, or a decreased level of expression, of said miRNA relative to a control reference value may be indicative of a viral infection.

[0054] According to one preferred embodiment, when assessing an activity of a quinoline derivative of formula (I) for treatment of a viral infection or when screening a drug candidate or vaccine candidate presumed effective in preventing and/or treating a viral infection, an observation of an induced or increased presence, or an increased level of expression, of said miRNA relative to a control reference value may be indicative of an activity of said quinoline derivative of formula (I) or of an efficacy of said drug candidate or vaccine candidate.

[0055] Uses and methods of the invention are carried out *in vitro* or *ex vivo*.

[0056] According to another of its objects, the invention relates to an *in vitro* or *ex vivo* use of an isolated nucleic acid probe able to specifically hybridize to miR-124, as a diagnostic agent for measuring a presence or a level expression of miR-124 for diagnosing a viral infection, in particular a retroviral infection, and more particularly an HIV infection, or for assessing an activity of a drug candidate or vaccine candidate presumed effective for preventing and/or treating a viral infection, in particular a retroviral infection, and more particularly an HIV infection.

[0057] The term "probe" as used herein, generally refers to a capture agent that is directed to a specific target miRNA biomarker sequence. Accordingly, each probe of a probe set has a respective target miRNA biomarker. A probe/target miRNA duplex is a structure formed by hybridizing a probe to its target miRNA biomarker.

[0058] An isolated nucleic acid probe suitable for the invention may be preferably a nucleic acid probe consisting in a nucleic acid sequence selected from the group consisting of SEQ ID NO: 6 to SEQ ID NO: 87.

[0059] According to one of its advantages, the invention provides a useful and reliable biomarker for the follow-up of patients infected with a virus, preferably with a retrovirus, and more preferably with an HIV virus.

[0060] According to one of its advantages, the invention provides a useful and reliable biomarker for the follow-up of patients infected with a virus, preferably with a retrovirus, and more preferably with an HIV virus, and treated with quinoline derivative of formula (I).

[0061] According to another of its advantages, the invention provides a sensible and dependable biomarker which may be easily used at the bed of a patient.

Uses and methods

[0062] According to one embodiment, use and methods according to the invention may, in particular, allow for the determining of a viral infection in a patient, and in particular for the follow-up of such infection.

[0063] According to one embodiment, a presence or a level of expression of miR-124 is measured into an isolated biological sample, and then is compared to a control reference value.

[0064] A modulation of the presence or level of expression of miR-124 relative to the control reference value may be indicative of a viral infection. In particular a reduced or suppressed presence, or a decreased level of expression, of said miRNA relative to a control reference value may be indicative of a viral infection.

[0065] According to another embodiment, the uses and methods of the invention may be for assessing an effectiveness of a treatment with said quinoline derivative of formula (I).

[0066] According to another embodiment, the uses and methods of the invention may be for assessing a therapeutic efficacy of quinoline derivatives of formula (I) as a therapeutic agent for preventing and/or treating a viral infection.

[0067] According to one embodiment, the uses and methods of the invention may be for assessing a patient compliance with a treatment with said quinoline derivative of formula (I).

[0068] The miR-124 biomarker may be used to monitor or manage quinoline derivatives of formula (I) activity during patient treatment of a viral infection, and in particular a retroviral infection, and more particularly an HIV infection or AIDS (Acquired Immune Deficiency Syndrome).

[0069] A method of assessing or monitoring the activity of a quinoline derivative of formula (I) in a patient treated with the quinoline derivative may involve measuring a level of expression of miR-124 in an isolated sample, preferably isolated PBMC (Peripheral Blood Mononuclear Cell), and comparing the measured level of expression to a level of expression of miR-124 in an isolated sample taken from the patient prior to the treatment. By following the miR-124 level, the activity of the quinoline derivative can be monitored over time.

[0070] According to one embodiment, a use or a method according to the invention may be implemented for optimizing the dosing regimen of a patient. Patients may respond differently to a given quinoline derivative of formula (I), depending on such factors as age, health, genetic background, presence of other complications, disease progression, and the co-administration of other drugs. It may be useful to utilize the miR-124 biomarker to assess and optimize the dosage regimen, such as the dose amount and/or the dose schedule, of a quinoline derivative in a patient. In this regard, miR-124-based biomarker can also be used to track and adjust individual patient treatment effectiveness over time. The biomarker can be used to gather information needed to make adjustments in a patient's treatment, increasing or decreasing the dose of an agent as needed. For example, a patient receiving a quinoline derivative can be tested using the miR-124 -based biomarker to see if the dosage is becoming effective, or if a more aggressive treatment plan needs to be put into place. The amount of administered drug, the timing of administration, the administration frequency, the duration of the administration may be then adjusted depending on the miR-124 biomarker measurement.

[0071] The miR-124 biomarker may also be used to track patient compliance during individual treatment regimes, or during clinical trials. This can be followed at set intervals to ensure that the patients included in the trial are taking the drugs as instructed. Furthermore, a patient receiving a quinoline derivative can be tested using the miR-124 biomarker to determine whether the patient complies with the dosing regimen of the treatment plan. An increased expression level of the biomarker compared to that of an untreated control sample is indicative of compliance with the protocol.

[0072] A biomarker of the invention may be implemented to assess and follow the efficacy of quinoline derivatives of formula (I). Accordingly, a presence or level of expression of miR-124 may be measured into an isolated biological sample obtained from a patient previously treated with a quinoline derivative of formula (I). Then, the measured presence or level expression of miR-124 into an isolated biological sample may be compared to a control reference value.

[0073] When an increase of the measured level relative to the control reference value is observed, then the measure is indicative of an activity of said quinoline derivatives of formula (I).

[0074] In another embodiment, when an increase of the measured level relative to the control reference value is observed, then the measure may be indicative of a responsiveness of a patient to a treatment with said quinoline derivatives of formula (I).

[0075] In another embodiment, when an increase of the measured level relative to the control reference value is observed, then the measure may be indicative of an effectiveness of a treatment with said quinoline derivatives of formula (I).

[0076] In another embodiment, when an increase of the measured level of expression relative to the control reference value is observed, then the measure may be indicative a therapeutic efficacy of said quinoline derivatives of formula (I) as a therapeutic agent for preventing and/or treating a viral infection.

[0077] When miR-124 from a sample is "increased" or "up-regulated" after a treatment with a quinoline derivative, as compared to a non-treated control reference value, this increase can be, for example, of about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 90%, 100%, 200%, 300%, 500%, 1,000%, 5,000% or more of the comparative control reference value (i.e., without the treatment by the quinoline derivative).

[0078] In particular, the measured level expression of miR-124 may be at least a two-fold, preferably at least a four-fold, preferably at least a six-fold, preferably at least an eight-fold, and more preferably at least a ten-fold increase relative

to said control reference value.

[0079] According to another embodiment of the invention, when monitoring a viral infection or assessing an efficacy of a viral infection treatment, in particular with a quinoline derivative of formula (I), a patient may be tested with a method or a use of the invention at a time interval selected from the group consisting of hourly, twice a day, daily, twice a week, weekly, twice a month, monthly, twice a year, yearly, and every other year. The then collected sample can be tested immediately, or can be stored for later testing.

[0080] According to another embodiment, use and methods according to the invention may, in particular, allow for the screening, identification or evaluation of potential active agents as a drug candidate.

[0081] In particular, use and methods according to the invention are particularly advantageous for the screening, identification or evaluation of potential active agents, such as a drug candidate or a vaccine presumed effective towards a viral infection.

[0082] According to another embodiment of the invention, a miR-124 biomarker may be implemented to screen a drug candidate or a vaccine candidate presumed effective for preventing and/or treating a viral infection. In such embodiment, a presence or level of expression of miR-124 may be measured into an isolated biological sample or isolated cell previously contacted with the drug or vaccine to be screened. Then, the obtained measure may be compared to a control reference value.

[0083] When an increase of the measured level into an isolated biological sample or isolated cell, previously contacted with the compound, drug or vaccine candidate to be screened, relative to a control reference value is observed, then the measure may be indicative of said candidate to have a biological effect and in particular to be efficient for altering the physiological activity of a cell.

[0084] In particular, a drug candidate or vaccine candidate may be characterized as being efficient in preventing and/or treating a viral infection, and in particular a retroviral infection, and more particularly an HIV infection.

[0085] When miR-124 from a sample is "increased" or "up-regulated" after treatment with a drug candidate or vaccine, as compared to a non-treated control reference value, this increase can be, for example, of about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 90%, 100%, 200%, 300%, 500%, 1,000%, 5,000% or more of the comparative control reference value (i.e., without the treatment by the quinoline derivative).

[0086] In particular, the measured level expression of miR-124 may be at least a two-fold, preferably at least a four-fold, preferably at least a six-fold, preferably at least an eight-fold, and more preferably at least a ten-fold increase relative to said control reference value.

[0087] The uses and methods of the invention may comprise measuring a level of expression of miR-124 into an isolated biological sample. Any suitable sample may be used to assess the miR-124 biomarker.

[0088] In particular, a biological sample suitable for the invention may be a biological fluid, such as a blood, a plasma, or a serum, a saliva, an interstitial fluid, or an urine sample; a cell sample, such as a cell culture, a cell line, or a PBMC sample, a tissue biopsy, such as an oral tissue, a gastrointestinal tissue, a skin, an oral mucosa sample, or a plurality of samples from a clinical trial. The sample can be a crude sample, or can be purified to various degrees prior to storage, processing, or measurement.

[0089] The step of collecting biological samples for the uses and methods of the invention is performed before carrying out the invention and is not a step of a use or a method in accordance with the invention.

[0090] Samples for miRNA assessment can be taken during any desired intervals. For example, samples can be taken hourly, twice per day, daily, weekly, monthly, every other month, yearly, or the like. The sample can be tested immediately, or can be stored for later testing.

[0091] The samples can be purified prior to testing. In some embodiments, the miR-124 can be isolated from the remaining cell contents prior to testing. Further, the miR-124 molecules can be separated from the rest of the mRNA in the sample, if desired. For example, the miR-124 can be separated from the mRNA based on size differences prior to testing.

[0092] Control reference value to be used for comparing the measured level of expression of miR-124 in a tested biological sample is obtained from a control sample.

[0093] Control samples can be taken from various sources. In some embodiments, control samples are taken from the patient prior to treatment or prior to the presence of the disease (such as an archival blood sample). In other embodiments, the control samples are taken from a set of normal, non-diseased members of a population. In another embodiment, a cell assay can be performed on a control cell culture, for example, that has not been treated with the test compound or has been treated with a reference compound, such as the 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine.

[0094] According to one embodiment, for the determination or monitoring of a viral infection in a patient, a control reference value may be obtained from an isolated biological sample obtained on an individual or group of individuals known to not suffer from such condition.

[0095] According to another embodiment, for the determination or monitoring of an efficacy of a treatment of a viral infection into a patient, a control reference value may be obtained from an isolated biological sample obtained from an

individual or group of individuals known to not suffer from such condition, and not receiving the treatment the efficacy of which is to be determined or monitored. Alternatively, a control reference value may be obtained from an isolated biological sample obtained from a patient suffering from a viral infection and receiving a treatment the efficacy of which being to be determined or monitored, the isolated biological sample being taken from the patient before administration of the treatment.

[0096] Numerous methods are available to the skilled man to measure a presence or level of expression of the miR-124 biomarker.

[0097] For example, nucleic acid assays or arrays can be used to assess the presence and/or expression level of miR-124 in a sample.

[0098] The sequence of the miR-124 may be used to prepare a corresponding nucleotide acting as complementary probe or primer to be used in different nucleic acid assays for detecting the expression or presence of the miR-124 biomarker in the sample, such as, but not limited to, Northern blots and PCR-based methods (e.g., Real-Time Reverse Transcription-PCR or qRT-PCR). Methods such as qRT-PCR may be used to accurately quantitate the amount of the miRNA in a sample.

[0099] Sense and anti-sense probes or primers according to the invention may be obtained using every process known to the man skilled in the art, in particular those that are described in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 3rd ED., 2001, Cold Spring Harbour, N. Y.).

[0100] Methods related to the detection and quantification of RNA or DNA are well known in the art. The man skilled in the art may for instance refer to Wang et al. (1989, Proc Natl Acad Sci USA, Vol. 86 : 917-921), de Wong et al. (2005, Bio Techniques, Vol. 39 (1): 75-85), de Nolan et al. (2006, Nat Protoc, Vol. 1(3) : 1559-1582) et de Klinck et al. (2008, Cancer Research, Vol. 68 : 657-663), or also to a general review published by Bustin (2000, Journal of Molecular Endocrinology, Vol. 25 : 169-193).

[0101] In one embodiment, a method for the detection and quantification of nucleic acids may be a fluorescent-dye-based method, wherein nucleic acid concentration is assessed by measuring the fluorescence intensity of ligands, such as dyes, that bind to said nucleic acids. Fluorescent dyes are well known in the art.

[0102] Alternatively, said nucleic acid may be quantified using spectrophotometry.

[0103] In another embodiment, a method for the detection and quantification of nucleic acids may be a hybridization-based method. Said hybridization-based methods may include PCR and quantitative-PCR (qRT-PCR or q-PCR) techniques or reverse transcriptase / polymerase based techniques. Advantageously, said method may comprise, or be further combined, with a sequencing step.

[0104] Those methods may comprise (i) a step of extraction of cellular mRNAs, (ii) a step of reverse transcription of mRNA to DNA using a reverse transcriptase and (iii) a step of DNA amplification from DNA obtained on the previous step. Usually, starting from the same sample, the following nucleic acids are amplified : (a) DNA obtained after a reverse transcription step of the target mRNA and (b) a DNA or a plurality of DNAs obtained after reverse transcription of mRNAs which are constitutively and constantly expressed by cells (« housekeeping genes »), such as RNAs coded by genes *MRPL19*, *PUM1* and *GADPH*.

[0105] The amplified DNA can be quantified, after separation by electrophoresis, and measure of DNA bands. Results related to the target mRNA(s) are expressed as relative units in comparison to mRNAs coded by « housekeeping » genes. In some embodiments, the step of separation of amplified DNAs is achieved after agarose gel electrophoresis, and then coloration of DNA bands with ethidium bromide, before quantification of DNA contained in those migration bands with densitometry. In other embodiments, one may use a micro-channel device in which amplified DNA is separated by capillar electrophoresis, before quantification of the emitted signal using a laser beam. Such a device may be a LabChip® device, for instance from the « GX » series, commercialized by the company Caliper LifeSciences (Hopkinton, MA, USA).

[0106] Quantitative results obtained by qRT-PCR can sometimes be more informative than qualitative data, and can simplify assay standardization and quality management. Thus, in some embodiments, qRT-PCR-based assays can be useful to measure miRNA levels during cell-based assays. The qRT-PCR method may be also useful in monitoring patient therapy. Commercially available qRT-PCR based methods {e.g., TaqmanR Array™}

[0107] Any suitable assay platform can be used to determine the expression or presence of the miRNA in a sample. For example, an assay may be in the form of a dipstick, a membrane, a chip, a disk, a test strip, a filter, a microsphere, a slide, a multiwell plate, or an optical fiber. An assay system may have a solid support on which an oligonucleotide corresponding to the miRNA is attached. The solid support may comprise, for example, a plastic, silicon, a metal, a resin, glass, a membrane, a particle, a precipitate, a gel, a polymer, a sheet, a sphere, a polysaccharide, a capillary, a film a plate, or a slide. The assay components can be prepared and packaged together as a kit for detecting an miRNA.

[0108] In some embodiments, an oligonucleotide array for testing for quinoline derivative or drug candidate activity in a biological sample can be prepared or purchased. An array typically contains a solid support and at least one oligonucleotide contacting the support, where the oligonucleotide corresponds to at least a portion of the miR-124 biomarker. In some embodiments, the portion of the miR-124 biomarker comprises at least 5, 10, 15, 20 or more bases.

[0109] According to one embodiment, the presence or expression of miR-124 may be assayed in combination with others miRNA also used as biomarkers. In such an embodiment, an array can be used to assess the expression or presence of multiple miRNAs in a sample, including miRNA-124. In general, the method comprises the following steps: a) contacting the sample with an array comprising a probe set under conditions sufficient for specific binding to occur; and b) examining the array to detect the presence of any detectable label, thereby evaluating the amount of the respective target miRNAs in the sample. The use of an expression array allows obtaining a miRNA expression profile for a given sample.

[0110] Methods of preparing assays or arrays for assaying miRNAs are well known in the art and are not needed to be further detailed here.

[0111] Nucleic acid arrays can be used to detect presence or differential expression of miRNAs in biological samples. Polynucleotide arrays (such as DNA or RNA arrays) typically include regions of usually different sequence polynucleotides ("capture agents") arranged in a predetermined configuration on a support. The arrays are "addressable" in that these regions (sometimes referenced as "array features") have different predetermined locations ("addresses") on the support of array. The region (i.e., a "feature" or "spot" of the array) at a particular predetermined location (i.e., an "address") on the array will detect a particular miRNA target. The polynucleotide arrays typically are fabricated on planar supports either by depositing previously obtained polynucleotides onto the support in a site specific fashion or by site specific in situ synthesis of the polynucleotides upon the support. Arrays to detect miRNA expression can be fabricated by depositing (e.g., by contact- or jet-based methods or photolithography) either precursor units (such as nucleotide or amino acid monomers) or pre-synthesized capture agent. After depositing the polynucleotide capture agents onto the support, the support is typically processed (e.g., washed and blocked for example) and stored prior to use.

[0112] An array to detect miRNA expression has at least two, three, four, or five different subject probes. However, in certain embodiments, a subject array may include a probe set having at least 10, at least 20, at least 50, at least 100, at least 200, at least 500, or at least 1,000 or more probes that can detect a corresponding number of miRNAs. In some embodiments, the subject arrays may include probes for detecting at least a portion or all of the identified miRNAs of an organism, or may include orthologous probes from multiple organisms.

[0113] A nucleic acid array may be contacted with a sample or labeled sample containing miRNA analytes under conditions that promote specific binding of the miRNA in the sample to one or more of the capture agents present on the array to exhibit an observed binding pattern. This binding pattern can be detected upon interrogating the array. For example, the target miRNAs in the sample can be labeled with a suitable label (such as a fluorescent compound), and the label then can be accurately observed (such as by observing the fluorescence pattern) on the array after exposure of the array to the sample. The observed binding pattern can be indicative of the presence and/or concentration of one or more miRNA components of the sample.

[0114] The labeling of miRNAs may be carried using methods well known in the art, such as using DNA ligase, terminal transferase, or by labeling the RNA backbone, etc. In some embodiments, the miRNAs may be labeled with fluorescent label. Exemplary fluorescent dyes include but are not limited to xanthene dyes, fluorescein dyes, rhodamine dyes, fluorescein isothiocyanate (FITC), 6 carboxyfluorescein (FAM), 6 carboxy-2',4',7',4',7-hexachlorofluorescein (HEX), 6 carboxy 4', 5' dichloro 2', 7' dimethoxyfluorescein (JOE or J), N,N,N',N' tetramethyl 6 carboxyrhodamine (TAMRA or T), 6 carboxy X rhodamine (ROX or R), 5 carboxyrhodamine 6G (R6G5 or G5), 6 carboxyrhodamine 6G (R6G6 or G6), and rhodamine 110; cyanine dyes, e.g. Cy3, Cy5 and Cy7 dyes; Alexa dyes, e.g. Alexa-fluor-555; coumarin, Diethyl-aminocoumarin, umbelliferone; benzimide dyes, e.g. Hoechst 33258; phenanthridine dyes, e.g. Texas Red; ethidium dyes; acridine dyes; carbazole dyes; phenoxazine dyes; porphyrin dyes; polymethine dyes, BODIPY dyes, quinoline dyes, Pyrene, Fluorescein Chlorotriazinyl, R1 10, Eosin, JOE, R6G, Tetramethylrhodamine, Lissamine, ROX, Naptho fluorescein, and the like.

[0115] In some embodiments, an oligonucleotide array for assessing immunomodulatory activity can be prepared or purchased, for example from Affymetrix. The array may contain a solid support and a plurality of oligonucleotides contacting the support. The oligonucleotides may be present in specific, addressable locations on the solid support; each corresponding to at least a portion of miRNA sequences which may be differentially expressed upon treatment of a quinoline derivative or a drug candidate in a cell or a patient. The miRNA sequences comprise at least one miR-124 sequence.

[0116] When an array is used to assess miRNAs, a typical method can contain the steps of 1) obtaining the array containing surface-bound subject probes; 2) hybridization of a population of miRNAs to the surface-bound probes under conditions sufficient to provide for specific binding (3) post-hybridization washes to remove nucleic acids not bound in the hybridization; and (4) detection of the hybridized miRNAs. The reagents used in each of these steps and their conditions for use may vary depending on the particular application.

[0117] Hybridization can be carried out under suitable hybridization conditions, which may vary in stringency as desired. Typical conditions are sufficient to produce probe/target complexes on an array surface between complementary binding members, i.e., between surface-bound subject probes and complementary miRNAs in a sample. In certain embodiments, stringent hybridization conditions may be employed. Hybridization is typically performed under stringent hybridization

conditions. Standard hybridization techniques which are well-known in the art (e.g. under conditions sufficient to provide for specific binding of target miRNAs in the sample to the probes on the array) are used to hybridize a sample to a nucleic acid array. Selection of appropriate conditions, including temperature, salt concentration, polynucleotide concentration, hybridization time, stringency of washing conditions, and the like will depend on experimental design, including source of sample, identity of capture agents, degree of complementarity expected, etc., and may be determined as a matter of routine experimentation for those of ordinary skill in the art. In general, a "stringent hybridization" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization are typically sequence dependent, and are different under different experimental conditions. Hybridization may be done over a period of about 12 to about 24 hours. The stringency of the wash conditions can affect the degree to which miRNA sequences are specifically hybridized to complementary capture agents. Those of ordinary skill will readily recognize that alternative but comparable hybridization and wash conditions can be utilized to provide conditions of similar stringency.

[0118] As an illustration, in one embodiment, the miRNA expression profiling experiments may be conducted using the Affymetrix Genechip miRNA Array 2.0 and following the protocols described in the instruction manual.

[0119] In one particular embodiment, said hybridization can be performed using the GeneChip® Hybridization, Wash, and Stain Kit (Affymetrix Ref. #900720). Advantageously, said hybridization is performed by following the protocols of the manufacturer.

[0120] After the miRNA hybridization procedure, the array-surface bound polynucleotides are typically washed to remove unbound nucleic acids. Washing may be performed using any convenient washing protocol, where the washing conditions are typically stringent, as described above. For instance, a washing step may be performed using washing buffers sold by the company Affymetrix (Ref. #900721 and #900722). The hybridization of the target miRNAs to the probes is then detected using standard techniques of reading the array. Reading the resultant hybridized array may be accomplished, for example, by illuminating the array and reading the location and intensity of resulting fluorescence at each feature of the array to detect miRNA/probe binding complexes.

miRNA-124

[0121] MicroRNAs (miRNAs) are small, single-stranded non-coding RNAs that can act in the cytoplasm of a cell to cause a decrease in the expression of their cognate target messenger RNAs or translation of the mRNA's protein product. Mature miRNAs are typically about 19-23 nucleotides in length. This ability of miRNAs to inhibit the production of their target proteins results in the regulation of many types of cellular activities, such as cell-fate determination, apoptosis, differentiation, and oncogenesis.

[0122] miR-124 was initially cloned in mouse. Human miR-124 precursor (or miRN-124 or miRNA-124 or micro RNA 124) was cloned from embryonic stem cells. 9 haplotypes of miR-124 precursors have been identified so far (Guo et al., PLoS ONE, 2009, 4(11):e7944), from which 3 are present in the Human, hsa-miR-124-1, hsa-miR-124-2 and hsa-miR-124-3. (SEQ ID NO:1 to SEQ ID NO:3).

[0123] The miR-124 microRNA precursor is a small non-coding RNA molecule. The mature ~21 nucleotide microRNAs are processed from hairpin precursor sequences by the Dicer enzyme. The mature sequences are SEQ ID NO: 4, UAAGGCACGCGGUGAAUGCC for miR-124-3' and SEQ ID NO: 5, CGUGUUCACAGCGGACCUUGAU for miR-124-5'.

[0124] miRNA-124 is preferentially expressed in brain, and could contribute to neurogenesis by downregulating SCP1 expression. Expression of miR124 in mouse neuronal cells induces a switch from general to neuron-specific alternative splicing by directly targeting the mRNA of PTBP1. miR-124 increases the abundance of neuron-specific PTBP2 and Gabbr1 mRNAs by preventing PTBP1-dependent exon skipping that leads to nonsense-mediated decay of these mRNAs.

[0125] At the point of mitotic exit within the vertebrate nervous system, when cells lose multipotency and begin to develop stable connections that will persist over life, a switch in ATP-dependent chromatin remodeling mechanisms occurs. This transition could be mediated by repression of BAF53A by miR9* (an miRNA processed from the opposite arm of the miR9 stem-loop precursor) and miR-124.

[0126] Experimental autoimmune encephalomyelitis (EAE) is a rodent model of multiple sclerosis characterized by inflammation of the central nervous system (CNS) associated with activation of resident macrophages in the CNS, or microglia, and infiltration of peripheral immune cells to the CNS. It has been found that miR-124 is as highly expressed in microglia and neurons. Expression of miR-124 is reduced in activated microglia during an EAE episode and in activated microglia in culture. Transfection of miR-124 deactivates bone marrow-derived macrophages, and intravenous administration of miR-124 inhibits development of lesions and reduced CNS inflammation in 3 mouse models of EAE. It has been found that miR-124 promotes microglia quiescence by deactivating macrophages via the CEBPA-PU. 1 pathway.

[0127] It has also been demonstrated that expression miR-124 in human fibroblasts induces their conversion into neurons. Further addition of neurogenic transcription factors ASCL1 and MYT1L enhances the rate of conversion and maturation of the converted neurons, whereas expression of these transcription factors without the aforementioned microRNA is ineffective.

[0128] An isolated nucleic acid probe suitable for measuring a presence or level expression of miR-124 is a nucleic

acid probe able to specifically hybridize to a miR-124, such as a precursor or a mature miR-124.

[0129] Such a nucleic acid probe may comprise from 18 to 30 nucleotides, in particular from 20 to 27, preferably from 20 to 25, preferably from 20, 22, or 25, and more preferably about 25 nucleotides. As previously indicated, such nucleic acid probes may be prepared according to any known methods in the art.

[0130] Methods and formulas are well known in the art to predict the optimal hybridization temperature for a given probe and a given target.

[0131] Thus, the man skilled in the art may easily calculate an optimal hybridization temperature based on a set of probes, on a given target sequence, and with particular conditions of hybridization.

[0132] Advantageously, the optimal hybridization temperature of said probes is between 40°C and 60°C, and more particularly between 45°C and 55°C, and preferably is about 48°C.

[0133] As examples of buffers useful for hybridizing a nucleic acid probe of the invention to a biomarker of the invention, one may mention, as an hybridization buffer, a buffer comprising 100mM MES, 1M [Na⁺], 20mM EDTA, 0.01% Tween-20, as a non-stringent washing buffer a buffer comprising 6X SSPE, 0.01% Tween-20, and as a stringent washing buffer a buffer comprising 100mM MES, 0.1M [Na⁺], 0.01% Tween-20.

[0134] A nucleic acid probe suitable for measuring a presence or level expression of miR-124 may, for instance, be a nucleic acid probe consisting in a nucleic acid sequence selected from the group consisting of SEQ ID NO: 6 to SEQ ID NO: 87.

[0135] A nucleic acid probe suitable for measuring a presence or level expression of the miR-124-1 precursor may, for instance, be a nucleic acid probe consisting in a nucleic acid sequence selected from the group consisting of SEQ ID NO: 6 to SEQ ID NO: 34, SEQ ID NO 86 and SEQ ID NO 87.

[0136] A nucleic acid probe suitable for measuring a presence or level expression of the miR-124-2 precursor may, for instance, be a nucleic acid probe consisting in a nucleic acid sequence selected from the group consisting of SEQ ID NO: 35 to SEQ ID NO: 65, SEQ ID NO 86 and SEQ ID NO 87.

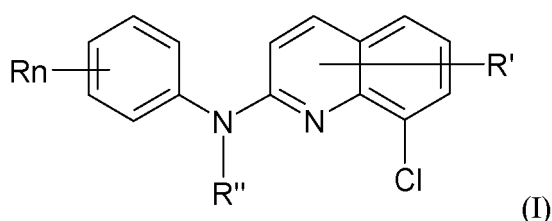
[0137] A nucleic acid probe suitable for measuring a presence or level expression of the miR-124-3 precursor may, for instance, be a nucleic acid probe consisting in a nucleic acid sequence selected from the group consisting of SEQ ID NO: 66 to SEQ ID NO: 85, SEQ ID NO 86 and SEQ ID NO 87.

[0138] A nucleic acid probe suitable for measuring a presence or level expression of a mature miR-124 may, for instance, be a nucleic acid probe consisting in a nucleic acid sequence selected from the group consisting of SEQ ID NO 86 and SEQ ID NO 87.

Quinoline derivatives

[0139] The quinoline derivatives useful for the invention may be quinoline derivatives efficient for treating a viral infection, such as the ones described in WO 2010/143169.

[0140] In particular, the quinoline derivatives useful for the invention are quinoline derivatives which may be represented by the following general formula (I):



wherein

- n is 1 or 2 and R, independently, represents a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₃) alkyl group; a -NR₁R₂ group in which R₁ and R₂ are independently a hydrogen atom or a (C₁-C₃) alkyl group; a (C₁-C₃) fluoroalkoxy group; a -NO₂ group; a phenoxy group; and a (C₁-C₄) alkoxy group,
- R' is a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₄) alkyl group and a (C₁-C₄) alkoxy group,
- R'' is a hydrogen atom or a (C₁-C₄) alkyl group,

or one of its pharmaceutically acceptable salt.

[0141] According to one embodiment R' and R'' are preferably a hydrogen atom.

[0142] According to another embodiment, a quinoline derivative suitable for the invention may be of formula (I), in which R independently, represents a halogen atom or a group chosen among a (C₁-C₃) alkyl group; a -NR₁R₂ group in

which R_1 and R_2 are independently a hydrogen atom or a (C₁-C₃) alkyl group; a (C₁-C₃) fluoroalkoxy group; and a (C₁-C₄) alkoxy group.

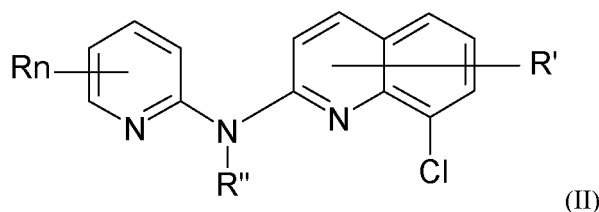
[0143] According to another embodiment, R independently, represents a fluorine or a chlorine atom or a group chosen among methyl or ethyl group, a -NH₂ group, a methoxy or ethoxy group, and a (C₁-C₃) fluoroalkoxy group.

[0144] According to another embodiment, n is preferably 1.

[0145] According to a preferred embodiment, a quinoline derivative suitable for the invention may be of formula (I), in which n is 1, R is a (C₁-C₃) fluoroalkoxy group, and R' and R'' are each a hydrogen atom.

[0146] According to a preferred embodiment, R is a methoxy, an ethoxy, or a propoxy group substituted with at least one fluorine atom. Preferably, R is a mono, bi ou trifluoromethoxy group.

[0147] Alternatively, the quinoline derivatives useful for the invention are quinoline derivatives which may be represented by a quinoline derivative of formula (II):



wherein:

- n is 1 or 2 and R, independently, represents a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₃) alkyl group; a -CN group; a hydroxyl group; a -COOR₁ group; a (C₁-C₃) fluoroalkyl group; a -NO₂ group; a -NR₁R₂ group with R₁ and R₂ being a hydrogen atom or a (C₁-C₃) alkyl group; and a (C₁-C₄) alkoxy group,
- R' is a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₄) alkyl group and a (C₁-C₄) alkoxy group,
- R'' is a hydrogen atom or a (C₁-C₄) alkyl group,

or one of its pharmaceutically acceptable salt.

[0148] According to one embodiment, a quinoline derivative suitable for the invention may be of formula (II), in which R' and R'' are preferably a hydrogen atom.

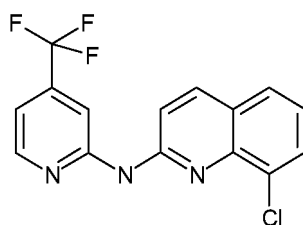
[0149] According to another embodiment, a quinoline derivative suitable for the invention may be of formula (II), in which R, independently, represents a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₃) alkyl group, a (C₁-C₃) fluoroalkyl group, a hydroxyl group, a -CN group, a -COOH group and a (C₁-C₃) alkoxy group.

[0150] According to another embodiment, a quinoline derivative suitable for the invention may be of formula (II), in which R, independently, represents a hydrogen atom, a halogen atom, a -CN group, a (C₁-C₃) alkyl group, a (C₁-C₃) fluoroalkyl group, and a hydroxyl group. According to another embodiment, a quinoline derivative suitable for the invention may be of formula (II), in which R, independently, represents a (C₁-C₃) fluoroalkyl group.

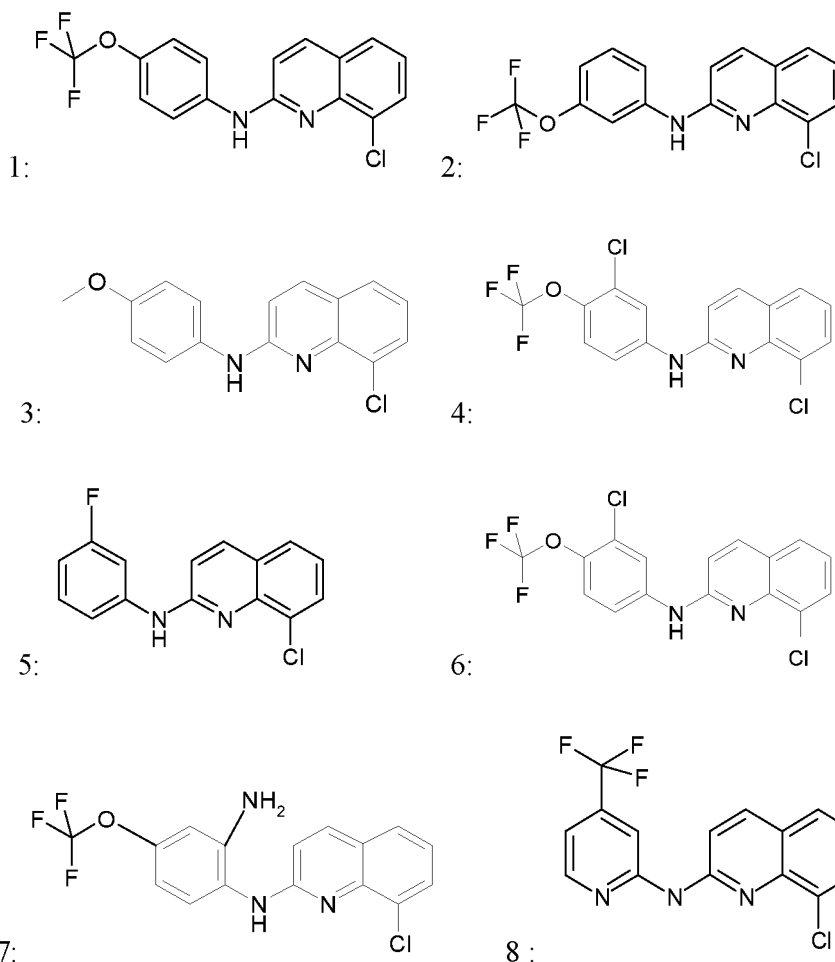
[0151] According to another embodiment, n is preferably 1.

[0152] According to a preferred embodiment, a quinoline derivative suitable for the invention may be of formula (II), in which n is 1, R is a (C₁-C₃) fluoroalkyl group, and R' and R'' are each a hydrogen atom.

[0153] Thus, according to said embodiment, a quinoline derivative may be represented by the following formula:



[0154] According to one embodiment, a quinoline derivative useful for the invention, or a salt thereof, may be selected from a group consisting of:



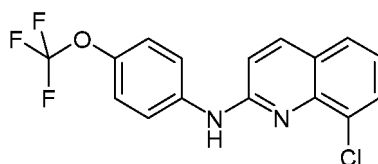
[0155] A pharmaceutically acceptable salt of a quinoline derivative of the invention, and more particularly of a compound having the general formula (I) or (II) according to the invention may be a salt of a compound having the general formula (I) or (II) and of an alkali metal, an alkaline earth metal, or an ammonium, comprising the salts obtained with organic ammonium bases, or salts of a compound having the general formula (I) or (II) and of organic or inorganic acid.

[0156] Salts more particularly suitable for the invention may be salts of sodium, potassium, calcium, magnesium, quaternary ammonium salts such as tetramethylammonium or tetraethylammonium, and addition salts with ammonia and pharmaceutically acceptable organic amines, such as methylamine, dimethylamine, trimethylamine, ethylamine, triethylamine, ethanolamine and tris(2-hydroxyethyl)amine.

[0157] Salts of a quinoline derivative of the invention, and more particularly of a compound having the general formula (I) or (II) and of inorganic acid suitable for the invention may be obtained with hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid or phosphoric acid.

[0158] Salts of a quinoline derivative of the invention, and more particularly of a compound having the general formula (I) or (II) and of organic acid suitable for the invention may be obtained with carboxylic acids and sulfonic acids such as formic acid, acetic acid, oxalic acid, citric acid, lactic acid, malic acid, succinic acid, malonic acid, benzoic acid, maleic acid, fumaric acid, tartaric acid, methanesulfonic acid, benzenesulfonic acid or p-toluenesulfonic acid.

[0159] According to a preferred embodiment, the quinoline derivative useful for the invention is 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine, which may be represented by the following formula:



[0160] The quinoline derivatives suitable for the invention may be prepared as described in WO 2010/143169.

[0161] The treatment can be oral or parenteral administration of a quinoline derivative. Suitable modes of administration and regimen are described in WO 2010/143169.

[0162] Any route of administration may be used. For example, a quinoline derivative can be administered by oral, parenteral, intravenous, transdermal, intramuscular, rectal, sublingual, mucosal, nasal, or other means. In addition, a quinoline derivative can be administered in a form of pharmaceutical composition and/or unit dosage form.

[0163] Suitable dosage forms include, but are not limited to, capsules, tablets (including rapid dissolving and delayed release tablets), powder, syrups, oral suspensions and solutions for parenteral administration.

[0164] The examples provided herein are intended to be merely exemplary, and those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, numerous equivalents of specific compounds, materials, and procedures. All such equivalents are considered to be within the scope of the invention and are encompassed by the appended claims.

EXAMPLES

Example 1

Modulation of miRNAs expression with 8-chloro-N-[4-(trifluoromethoxy)phenyl] quinolin-2-amine

Materials & Methods

[0165] In the context of HIV-1 inhibition by the quinoline derivative, 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine, it has been studied if the treatment could modulate host miRNAs expression.

[0166] For that purpose, peripheral blood mononuclear cells (PBMCs) of five healthy donors have been isolated by centrifugation on a FICOLL gradient. The cells have then been cultivated at 37°C, 5% CO₂ to a density of 1x10⁶ cells/mL in RPMI Glutamax medium (Life Technologies Ref 61870-010) supplemented with 10% fetal calf serum (FCS) (Thermo Fischer Ref SV30160.03) 1000 U/mL of IL2 (Peprotech Ref 200-02) and 5 µg/mL of PHA (Roche ref 1249738). Three days later, cells have been pooled and resuspended to a density of 1x10⁶ cells/mL in RPMI Glutamax medium supplemented with 10% fetal calf serum (FCS) 1000 U/mL of IL-2 and distributed in 12 wells plates (Falcon Ref 353043) with 1.2 mL/well (4 wells per condition).

[0167] HIV-1 infection has been performed with 1 ng of Ada-M R5 HIV strain/well. Cells were treated for 6 days with 1.2 mL/well of 60 µM solution of 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine or with 0.12% DMSO (Sigma Ref D4818 as negative control).

[0168] Cells were then pooled by conditions, centrifuged, and pellets were resuspended in 700 µL of Qiazol lysis buffer (Qiagen Ref 217004) for miRNeasy kit extraction from Qiagen (Qiagen Ref 217004). RNAs were extracted according to manufacturer's instructions. Extracted RNAs quality and amount were controlled using Agilent Bioanalyzer 2100 and Nanodrop spectrophotometry ND-1000. Mean RIN value was 8.84 (from 7.2 to 9.7). A total RNA amount of 90 ng per sample was labeled using FlashTag™ Biotin HSR RNA Labeling Kit (901911) and hybridized overnight to the Affymetrix Genechip miRNA Array 2.0. (901753) The arrays were washed and stained using standard Affymetrix protocol and scanned using the Affymetrix Scanner. Quality controls were performed using Expression Console metrics from Affymetrix (version 1.2).

[0169] Data were normalized using Expression Console "RMA+DABG" normalization method and a miRNA was considered expressed if the corresponding DABG P-Value was lesser or equal to 0.05. A miRNA was considered expressed in one condition if miRNA was expressed in at least 75% of the PBMCs donors of this condition. A paired Student's t-test was applied on expressed miRNAs that were considered differentially expressed between two conditions if fold-change was greater or equal to 1.5 and T-Test P-Value was lesser or equal to 0.05.

Results

[0170] Comparison of miRNA expression between infected and non-infected cells highlighted multiple modification (up or down-regulation) resulting from HIV-1 infection. In particular it is observed that miRNA-124 was down-regulated in HIV infected PBMCs.

[0171] In contrast, comparison between 8-chloro-N-[4-(trifluoromethoxy)phenyl] quinolin-2-amine treated or untreated HIV infected PBMCs highlighted only one miRNA, miR-124, whose expression was confidently increased (about 13 fold) under treatment.

[0172] Accordingly, miR-124 is validated as being a relevant biomarker to monitor the efficacy of quinoline derivatives according to the invention as anti-viral drugs in AIDS patient, and in particular the 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine.

Example 2

Evaluation of the efficiency of quinoline derivatives on the expression of miR-124 3p.

[0173] In addition to Example 1 which provides an assessment of mi-RNA expression in the context of HIV-1 infection, Example 2 assesses the variation of miR-124 expression in the absence of HIV-1. The screening method was tested to evaluate a set of quinoline derivatives and known antiretroviral drugs such as Maraviroc, Efavirenz, Darunavir and azidothymidine (AZT).

Materials & Methods

Extraction of PBMC using a FICOLL™ gradient

[0174] For that purpose, Peripheral blood mononuclear cells (PBMCs) of four healthy donors have been isolated by centrifugation on a FICOLL™ gradient according to standard protocols.

[0175] Briefly, 60-70 mL of buffy-coat are poured in a flask of 175 cm², and the volume is adjusted to 300 mL using PBS in order to obtain a dilution of about 5-fold of the buffy coat. 38 mL of diluted Buffy are then added to Falcon™ tubes of 50 mL comprising 12 mL of FICOLL™ (Histopack-1077) at ambient temperature. The preparation is centrifuged for 30 minutes at 1600 rpm (=515 rcf) at ambient temperature. The lymphocyte ring is recovered from the Falcon™ tube with a transfer pipette (Pastette®) and then washed with PBS using centrifugation for 10 minutes at 1200 rpm (= 290 rcf) and at ambient temperature until the supernatant becomes clear.

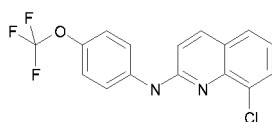
[0176] The cells are then resuspended at 37°C to a density of 1,5x10⁶ cells/mL in RPMI Glutamax medium (Life Technologies Ref 61870-010) supplemented with 10% fetal calf serum (FCS) (Thermo Fischer Ref SV30160.03) and without activation. Cells are incubated for 48 hours at 37°C under 5% CO₂.

Treatment of cells with screened molecules

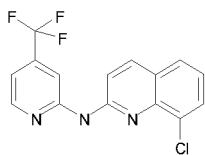
[0177] Six-well plates are used for the screening. Within each well comprising 3.10⁶ cell/4 ml RPMI supplemented with 10% fetal calf serum and 40 U/mL IL-2 (Peprotech Ref 200-02) are added screened molecules. 100% DMSO (0,8 µL) is added to the well and tested as a negative control.

[0178] Each tested condition is set up as described herebelow and the final corresponding volume is adjusted accordingly in the well:

1) Quinoline derivatives : (8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine and 8-chloro-N-[4-(trifluoromethyl)pyridin-2-yl]quinolin-2-amine - Respectively compounds 1 and 8) in 100% DMSO - (5 µM and final volume 0,4µL) :



« Compound 1 » (5 µM)



« Compound 8 » (5 µM)

2) Other antiretroviral drugs : Maraviroc, Efavirenz, Darunavir, AZT (10 µM for all - final volume 0,8µL).

[0179] The wells are incubated for three days at 37°C under 5% CO₂. Medium is changed (Day 3) according to standard protocols. Briefly, plates are centrifuged at 1200 rpm for 5 minutes and 3 mL of supernatant is removed. 3 mL of RPMI supplemented with 10% fetal calf serum and 40 U/mL IL-2 is then added with 0,6 µL (for 10 µM final concentration) or 0,3 µL (for 5 µM final concentration) of a stock solution of screened molecule at 50 mM in 100% DMSO or 0,6 µL of 100% DMSO as a negative control.

Extraction of miRNAs (Day 6)

[0180] Cells are recovered within Falcon™ tubes of 15 mL, centrifugated at 1200 rpm for 5 minutes, and then washed in 10 mL PBS and further centrifugated at 1200 rpm for 5 minutes. Cells are then resuspended in 1 mL PBS and counted.

[0181] 6×10^6 cells are recovered and centrifugated at 1200 rpm for 5 minutes. The cell pellet is lysed in 300 μ L of ML lysis buffer from the Macherey Nagel Nucleospin® miRNA extraction kit (*Macherey Nagel Ref 740971*), and further stored at -20°C.

[0182] 5 μ L of 2×10^8 copies / μ L of spike-in control (Ce_miR-39 from QIAGEN® - *reference 219610, sequence 5' UCACCGGGUGUAAAUCAGCUUG 3'*) are added for each sample. The miRNA extraction is achieved using the protocol from Macherey Nagel Nucleospin® miRNA extraction kit using an elution volume for RNAs of 50 μ L and miRNAs of 30 μ L, and further stored at -20°C.

Reverse transcription of miRNAs (Day 6)

[0183] The reverse transcription step is followed for 12 μ L of miRNA using the miScript RT II reverse transcription (RT) kit from QIAGEN® using the miScript HiSpec buffer, and further stored at -20°C.

Quantitative PCR of miRNAs (Day 6)

[0184] The quantitative PCR step is achieved using the QIAGEN® miScript SYBR® Green PCR kit and miScript Primer Assays according to the manufacturer's protocol.

Composition of the miScript reaction mix for 384-well plates:

[0185]

Mix	μ L/reaction
2X SYBR® Green mix	5
10X Universal Primer	1
10X Primer Assay	1
H ₂ O	2
Total Mix volume:	9
Template cDNA in H ₂ O (*)	1
Final volume:	10
(*) cDNA prepared using the miScript II RT kit	

[0186] The reaction is repeated in triplicates in a 384-well plate according to the manufacturer's protocol on a Light-Cycler® 380 Roche Real-Time PCR system. Cycling conditions are also set up according to the manufacturer's protocol:

Step	Time	Temperature
<u>Initial activation step 3-step cycling:</u>	15 min	95°C
Denaturation	15 s	94°C
Annealing	30 s	55°C
Extension	30 s	70°C
Cycle number	40 cycles	

[0187] Relative and Absolute quantification of qPCR are known techniques in the Art and can be achieved as further detailed below.

1) Relative quantification

[0188] From a dilution to the $1/10^{\text{th}}$ in H₂O for the miR-124 qPCR (Hs_miR-124a) or to the $1/100^{\text{th}}$ for reference/house-keeping gene qPCR (Hs_miR-26a and Hs_miR-191, using miScript Primer Assays (Hs_miR-124a, Hs_miR-26a and Hs_miR-191).

[0189] The analysis is achieved using relative quantification models without efficiency correction ($2^{-\Delta\Delta C_p}$) using the

average of crossing points (Cp) values from triplicates of miR-124 and the average of the average of triplicates of miR-26a and miR-191.

2) Absolute quantification

[0190] From a dilution to the 1/10th in H₂O for the miR-124 qPCR and miScript Primer Assays (Hs_miR-124a et Ce_miR-39). Calibration curves are achieved according to standard protocols. The analysis is achieved by normalizing the average of miR-124 triplicates with the average of miR-39 triplicates and further normalizing with the number of cells.

Results

[0191] Results show good agreement between Relative and Absolute quantification for all molecules. DMSO control samples have a fold-change of 1, meaning no variation in miR-124a expression. All the tested quinoline derivatives show a modulation of miR-124 corresponding to a ten-fold increase of the expression of miR-124.

[0192] In comparison, other antiretroviral drugs do not induce significant modulation of the expression of miR-124.

[0193] Thus, this Example shows that the miR-124 is a suitable biomarker for screening a drug candidate or vaccine candidate presumed effective in preventing and/or treating a viral infection. It is also particularly useful for assessing the activity of a quinoline derivative of the invention.

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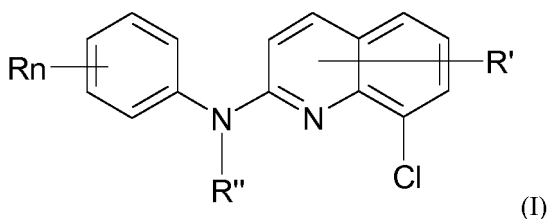
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Claims

1. An *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker of a viral infection, or of an efficacy of a therapeutic treatment of said viral infection.
2. The use according to claim 1, wherein a measured level of expression of said miR-124 into an isolated biological sample is compared to a control reference value, and wherein a modulation of said measured level relative to said control reference value is indicative of a viral infection, or of an efficacy of a therapeutic treatment of said viral infection.
3. The use according to claim 1, wherein the efficacy of a therapeutic treatment of said viral infection is determined by assessing the biological effect of a candidate compound, to alter the physiological activity of a protein or a cell.
4. An *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker for screening a drug candidate or vaccine candidate, in particular a quinoline derivative, presumed effective in preventing and/or treating a viral infection.
5. The use according to the preceding claims 3 or 4, wherein a measured level of expression of said miR-124 into an isolated biological sample in presence of said candidate compared to a control reference value, and wherein a modulation of said measured level relative to said control reference value is indicative of the biological effect of said candidate, and in particular of the efficacy of said drug candidate or vaccine candidate in preventing and/or treating a viral infection.
6. The use according to claims 2 or 5, wherein said biological sample is selected in a group consisting of a biological tissue sample, a whole blood sample, a swab sample, a plasma sample, a serum sample, a saliva sample, a vaginal fluid sample, a sperm sample, a pharyngeal fluid sample, a bronchial fluid sample, a fecal fluid sample, a cerebrospinal fluid sample, a lacrymal fluid sample and a tissue culture supernatant sample.
7. The use according to claims 3 to 6, wherein said drug candidate or vaccine candidate is a quinoline derivative of formula (I):

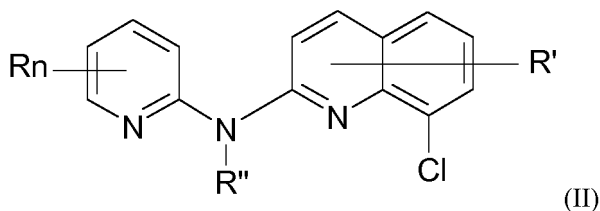


wherein

- n is 1 or 2 and R, independently, represents a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₃) alkyl group; a -NR₁R₂ group in which R₁ and R₂ are independently a hydrogen atom or a (C₁-C₃) alkyl group; a (C₁-C₃) fluoroalkoxy group; a -NO₂ group; a phenoxy group; and a (C₁-C₄) alkoxy group,
- R' is a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₄) alkyl group and a (C₁-C₄) alkoxy group,
- R'' is a hydrogen atom or a (C₁-C₄) alkyl group,

or one of its pharmaceutically acceptable salt.

8. The use according to claims 3 to 6, wherein said drug candidate or vaccine candidate is a quinoline derivative of formula (II):



wherein

- n is 1 or 2 and R, independently, represents a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₃) alkyl group; a -CN group; a hydroxyl group; a -COOR₁ group; a (C₁-C₃)fluoroalkyl group; a -NO₂ group; a -NR₁R₂ group with R₁ and R₂ being a hydrogen atom or a (C₁-C₃)alkyl group; and a (C₁-C₄) alkoxy group,
- R' is a hydrogen atom, a halogen atom or a group chosen among a (C₁-C₄) alkyl group and a (C₁-C₄) alkoxy group,
- R'' is a hydrogen atom or a (C₁-C₄) alkyl group,

or one of its pharmaceutically acceptable salt.

9. An *in vitro* or *ex vivo* use of at least one miRNA, said at least one miRNA being miR-124, as a biomarker of an activity of a quinoline derivative or one of its pharmaceutically acceptable salt, on a viral infection.
10. The use according to claim 9, wherein said quinoline derivative is of formula (I) as defined in claim 7, or of formula (II) as defined in claim 8, or one of its pharmaceutically acceptable salt, for example 8-chloro-N-[4-(trifluoromethoxy)phenyl]quinolin-2-amine or 8-chloro-N-[4-(trifluoromethyl)pyridin-2-yl]quinolin-2-amine.
11. The use according to any one of claims 9 or 10, wherein a measured level expression into an isolated biological sample is compared to a control reference value, and wherein an increase of said measured level relative to said control reference value is indicative of an activity of said quinoline derivative.
12. An *in vitro* or *ex vivo* method for assessing a viral infection in a patient presumed to be infected with a virus, comprising at least the steps of:

- a- measuring a presence or an expression level of at least one miRNA, said at least one miRNA being miR-124, in a biological sample previously obtained from said patient; and
- b- comparing said presence or expression level to a control reference value,

wherein a modulated presence or level of expression of said miRNA relative to said control reference value is indicative of a viral infection.

13. An *in vitro* or *ex vivo* method of assessing an activity of a quinoline derivative according to anyone of claims 9 or 10 for preventing and/or treating a viral infection in a patient treated with said quinoline derivative, comprising at least the steps of:

- a- measuring a presence or an expression level of at least one miRNA, said at least one miRNA being miR-124, in a first biological sample previously obtained from said patient before administering said quinoline derivative and in a second biological sample previously obtained from said patient after administering said quinoline derivative; and
- b- determining if said presence or expression level is modulated in the second biological sample obtained after the treatment as compared to the second biological sample obtained before the treatment;

wherein a modulated presence or level of expression of said miRNA is indicative of an activity of said quinoline derivative.

14. An *in vitro* or *ex vivo* method for screening a drug candidate or vaccine candidate presumed effective in preventing and/or treating a viral infection, comprising at least the steps of:

- a- treating at least one isolated cell able to express at least one miRNA, said at least one miRNA being miR-124, with said candidate, said cell being under conditions suitable for expressing said at least one miRNA,
- b- measuring a presence or expression level of said at least one miRNA,
- c- comparing said measured presence or expression level with a measure or expression level of said at least one miRNA in an untreated isolated cell,

wherein a modulated presence or level of expression of said miRNA is indicative of the efficacy of said drug candidate or vaccine candidate on a viral infection.

15. An *in vitro* or *ex vivo* use of an isolated nucleic acid probe able to specifically hybridize to miR-124, as a diagnostic agent for measuring a presence or a level expression of miR-124, for diagnosing a viral infection or for assessing an activity of a drug candidate or vaccine candidate for preventing and/or treating a viral infection.

16. The use according to claim 15, wherein said nucleic acid probe consists in a nucleic acid sequence selected from the group consisting of SEQ ID NO: 6 to SEQ ID NO: 87.

Patentansprüche

1. In-vitro- oder Ex-vivo-Verwendung mindestens einer miRNA, wobei es sich bei der mindestens einen miRNA um miR-124 handelt, als Biomarker für eine Virusinfektion oder für die Wirksamkeit einer therapeutischen Behandlung der Virusinfektion.

2. Verwendung nach Anspruch 1, wobei ein gemessenes Niveau der Expression der miR-124 in einer isolierten biologischen Probe mit einem Kontrollvergleichswert verglichen wird und wobei eine Modulation des gemessenen Niveaus im Vergleich zu dem Kontrollvergleichswert indikativ für eine Virusinfektion ist, oder für die Wirksamkeit einer therapeutischen Behandlung der Virusinfektion.

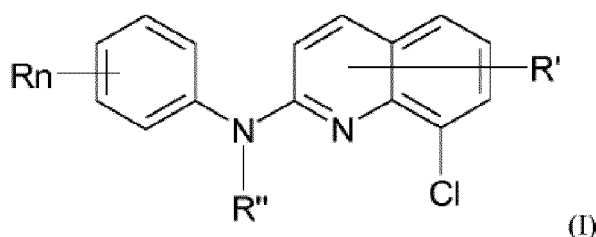
3. Verwendung nach Anspruch 1, wobei die Wirksamkeit einer therapeutischen Behandlung der Virusinfektion durch Untersuchung der biologischen Wirkung einer Kandidatenverbindung auf die Veränderung der physiologischen Aktivität eines Proteins oder einer Zelle bestimmt wird.

4. In-vitro- oder Ex-vivo-Verwendung mindestens einer miRNA, wobei es sich bei der mindestens einen miRNA um miR-124 handelt, als Biomarker zur Durchmusterung eines Arzneimittelkandidaten oder Impfstoffkandidaten, insbesondere eines Chinolinderivats, von dem vermutet wird, dass er bei der Prävention und/oder Behandlung einer Virusinfektion effektiv ist.

5. Verwendung nach dem vorhergehenden Anspruch 3 oder 4, wobei ein gemessenes Niveau der Expression der miR-124 in einer isolierten biologischen Probe in Gegenwart des Kandidaten mit einem Kontrollvergleichswert verglichen wird und wobei eine Modulation des gemessenen Niveaus im Vergleich zu dem Kontrollvergleichswert indikativ für die biologische Wirkung des Kandidaten und insbesondere für die Wirksamkeit des Arzneimittelkandidaten bzw. Impfstoffkandidaten bei der Prävention und/oder Behandlung einer Virusinfektion ist.

6. Verwendung nach Anspruch 2 oder 5, wobei die biologische Probe aus der aus einer biologischen Gewebeprobe, einer Vollblutprobe, einer Abstrichprobe, einer Plasmaprobe, einer Serumprobe, einer Speichelprobe, einer Scheidenflüssigkeitsprobe, einer Spermaprobe, einer Rachenflüssigkeitsprobe, einer Bronchialflüssigkeitsprobe, einer Fäkalflüssigkeitsprobe, einer Zerebrospinalflüssigkeitsprobe, einer Tränenflüssigkeitsprobe und einer Gewebekulturüberstandsprobe bestehenden Gruppe ausgewählt ist.

7. Verwendung nach den Ansprüchen 3 bis 6, wobei es sich bei dem Arzneimittelkandidaten bzw. Impfstoffkandidaten um ein Chinolinderivat der Formel (I) handelt:

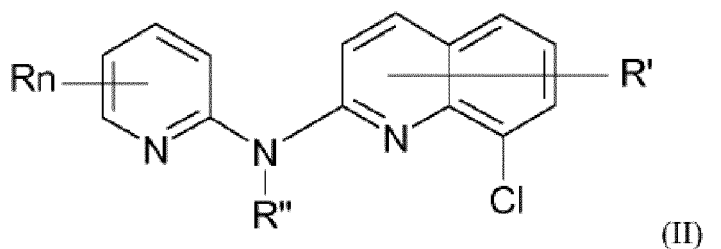


wobei

- n für 1 oder 2 steht und R unabhängig für ein Wasserstoffatom, ein Halogenatom oder eine aus einer (C₁-C₃)-Alkylgruppe, einer -NR₁R₂-Gruppe, in welcher R₁ und R₂ unabhängig voneinander für ein Wasserstoffatom oder eine (C₁-C₃)-Alkylgruppe stehen, einer (C₁-C₃)-Fluoralkoxygruppe, einer -NO₂-Gruppe, einer Phenoxygruppe und einer (C₁-C₄)-Alkoxygruppe ausgewählte Gruppe steht,
- R' für ein Wasserstoffatom, ein Halogenatom oder eine aus einer (C₁-C₄)-Alkylgruppe und einer (C₁-C₄)-Alkoxygruppe ausgewählte Gruppe steht,
- R'' für ein Wasserstoffatom oder eine (C₁-C₄)-Alkylgruppe steht,

oder ein pharmazeutisch unbedenkliches Salz davon.

8. Verwendung nach den Ansprüchen 3 bis 6, wobei es sich bei dem Arzneimittelkandidaten bzw. Impfstoffkandidaten um ein Chinolinderivat der Formel (II) handelt:



wobei

- n für 1 oder 2 steht und R unabhängig für ein Wasserstoffatom, ein Halogenatom oder eine aus einer (C₁-C₃)-Alkylgruppe, einer -CN-Gruppe, einer Hydroxylgruppe, einer -COOR₁-Gruppe, einer (C₁-C₃)-Fluoralkylgruppe, einer -NO₂-Gruppe, einer -NR₁R₂-Gruppe, in welcher R₁ und R₂ für ein Wasserstoffatom oder eine (C₁-C₃)-Alkylgruppe stehen, und einer (C₁-C₄)-Alkoxygruppe ausgewählte Gruppe steht,
- R' für ein Wasserstoffatom, ein Halogenatom oder ein aus einer (C₁-C₄)-Alkylgruppe und einer (C₁-C₄)-Alkoxygruppe ausgewählte Gruppe steht,
- R'' für ein Wasserstoffatom oder eine (C₁-C₄)-Alkylgruppe steht,

oder ein pharmazeutisch unbedenkliches Salz davon.

9. In-vitro- oder Ex-vivo-Verwendung mindestens einer miRNA, wobei es sich bei der mindestens einen miRNA um miR-124 handelt, als Biomarker für eine Aktivität eines Chinolinderivats oder eines pharmazeutisch unbedenklichen Salzes davon auf eine Virusinfektion.
10. Verwendung nach Anspruch 9, wobei das Chinolinderivat die wie in Anspruch 7 definierte Formel (I) oder die wie in Anspruch 8 definierte Formel (II) aufweist, oder ein pharmazeutisch unbedenkliches Salz davon, zum Beispiel 8-Chlor-N-[4-(trifluormethoxy)phenyl]chinolin-2-amin oder 8-Chlor-N-[4-(trifluormethyl)pyridin-2-yl]chinolin-2-amin.
11. Verwendung nach einem der Ansprüche 9 oder 10, wobei ein gemessenes Niveau einer Expression in eine isolierte biologische Probe mit einem Kontrollvergleichswert verglichen wird und wobei eine Erhöhung des gemessenen Niveaus im Vergleich zu dem Kontrollvergleichswert indikativ für eine Aktivität des Chinolinderivats ist.

12. In-vitro- oder Ex-vivo-Verfahren zur Untersuchung einer Virusinfektion in einem Patienten, von dem vermutet wird, dass er mit einem Virus infiziert ist, wobei das Verfahren wenigstens die folgenden Schritte umfasst:

a- das Messen eines Vorhandenseins oder eines Expressionsniveaus mindestens einer miRNA, wobei es sich bei der mindestens einen miRNA um miR-124 handelt, in einer zuvor von dem Patienten erhaltenen biologischen Probe, und
b- den Vergleich des Vorhandenseins oder Expressionsniveaus mit einem Kontrollvergleichswert,

wobei ein moduliertes Vorhandensein oder Expressionsniveau der miRNA im Vergleich zu dem Kontrollvergleichswert indikativ für eine Virusinfektion ist.

13. In-vitro- oder Ex-vivo-Verfahren zur Untersuchung einer Aktivität eines Chinolinderivats nach einem der Ansprüche 9 oder 10 zur Prävention und/oder Behandlung einer Virusinfektion in einem mit dem Chinolinderivat behandelten Patienten, welches mindestens die folgenden Schritte umfasst:

a- das Messen eines Vorhandenseins oder eines Expressionsniveaus mindestens einer miRNA, wobei es sich bei der mindestens einen miRNA um miR-124 handelt, in einer zuvor von dem Patienten vor der Verabreichung des Chinolinderivats erhaltenen ersten biologischen Probe und in einer zuvor von dem Patienten nach Verabreichung des Chinolinderivats erhaltenen zweiten biologischen Probe, und
b- die Feststellung, ob das Vorhandensein bzw. das Expressionsniveau in der zweiten, nach der Behandlung erhaltenen biologischen Probe im Vergleich zur zweiten, vor der Behandlung erhaltenen Probe moduliert ist,

wobei ein moduliertes Vorhandensein oder Expressionsniveau der miRNA indikativ für eine Aktivität des Chinolinderivats ist.

14. In-vitro- oder Ex-vivo-Verfahren zum Durchmustern eines Arzneimittelkandidaten oder Impfstoffkandidaten, von dem vermutet wird, dass er bei der Prävention und/oder Behandlung einer Virusinfektion effektiv ist, welches mindestens einen der folgenden Schritte umfasst:

a- das Behandeln mindestens einer zur Expression mindestens einer miRNA fähigen isolierten Zelle, wobei es sich bei der mindestens einen miRNA um miR-124 handelt, mit dem Kandidaten, wobei die Zelle unter für die Expression der mindestens einen miRNA geeigneten Bedingungen gehalten wird,
b- das Messen eines Vorhandenseins oder Expressionsniveaus der mindestens einen miRNA,
c- das Vergleichen des gemessenen Vorhandenseins bzw. Expressionsniveaus mit einem Maß oder Expressionsniveau der mindestens einen miRNA in einer unbehandelten isolierten Zelle,

wobei ein moduliertes Vorhandensein oder Expressionsniveau der miRNA indikativ für die Wirksamkeit des Arzneimittelkandidaten bzw. Impfstoffkandidaten auf eine Virusinfektion ist.

15. In-vitro- oder Ex-vivo-Verwendung einer zum spezifischen Hybridisieren mit miR-124 fähigen isolierten Nukleinsäuresonde als Diagnostikum zum Messen eines Vorhandenseins oder eines Expressionsniveaus von miR-124, zur Diagnose einer Virusinfektion oder zur Untersuchung einer Aktivität eines Arzneimittelkandidaten oder Impfstoffkandidaten bei der Prävention und/oder Behandlung einer Virusinfektion.

16. Verwendung nach Anspruch 15, wobei die Nukleinsäuresonde aus einer aus der aus SEQ ID NO: 6 bis SEQ ID NO: 87 bestehenden Gruppe ausgewählten Nukleinsäuresequenz besteht.

Revendications

1. Utilisation *in vitro* ou *ex vivo* d'au moins un miARN, ledit au moins un miARN étant miR-124, en tant que biomarqueur d'une infection virale, ou d'une efficacité d'un traitement thérapeutique de ladite infection virale.
2. Utilisation selon la revendication 1, dans laquelle un taux d'expression mesuré dudit miR-124 dans un échantillon biologique isolé est comparé à une valeur de référence témoin, et dans laquelle une modulation dudit taux mesuré par rapport à ladite valeur de référence témoin est indicative d'une infection virale, ou d'une efficacité d'un traitement thérapeutique de ladite infection virale.

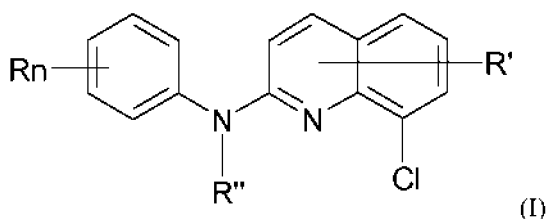
3. Utilisation selon la revendication 1, dans laquelle l'efficacité d'un traitement thérapeutique de ladite infection virale est déterminée par évaluation de l'effet biologique d'un composé candidat, pour modifier l'activité physiologique d'une protéine ou une cellule.

4. Utilisation *in vitro* ou *ex vivo* d'au moins un miARN, ledit au moins un miARN étant miR-124, en tant que biomarqueur pour cribler un médicament candidat ou un vaccin candidat, en particulier un dérivé de quinoléine, présumé être efficace dans la prévention et/ou le traitement d'une infection virale.

5. Utilisation selon les revendications précédentes 3 ou 4, dans laquelle un taux d'expression dudit miR-124 est mesuré dans un échantillon biologique isolé en présence dudit candidat par rapport à une valeur de référence témoin, et dans laquelle une modulation dudit taux mesuré par rapport à ladite valeur de référence témoin est indicative de l'effet biologique dudit candidat et, en particulier, de l'efficacité dudit médicament candidat ou vaccin candidat dans la prévention et/ou le traitement d'une infection virale.

6. Utilisation selon les revendications 2 ou 5, dans laquelle ledit échantillon biologique est choisi dans un groupe constitué d'un échantillon de tissu biologique, un échantillon de sang total, un échantillon d'écouvillon, un échantillon de plasma, un échantillon de sérum, un échantillon de salive, un échantillon de fluide vaginal, un échantillon de sperme, un échantillon de fluide pharyngé, un échantillon de fluide bronchique, un échantillon de fluide fécal, un échantillon de liquide céphalorachidien, un échantillon de fluide lacrymal et un échantillon de surnageant de culture de tissu.

7. Utilisation selon les revendications 3 à 6, dans laquelle ledit médicament candidat ou vaccin candidat est un dérivé de quinoléine de formule (1) :

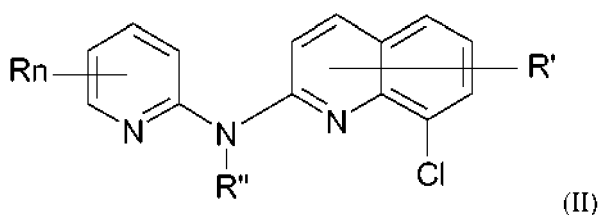


dans lequel

- n est 1 ou 2 et R, indépendamment, représente un atome d'hydrogène, un atome d'halogène ou un groupe choisi parmi un groupe alkyle en (C₁-C₃) ; un groupe -NR₁R₂ dans lequel R₁ et R₂ sont indépendamment un atome d'hydrogène ou un groupe alkyle en (C₁-C₃) ; un groupe fluoroalkoxy en (C₁-C₃) ; un groupe -NO₂ ; un groupe phénoxy ; et un groupe alkoxy en (C₁-C₄),
- R' est un atome d'hydrogène, un atome d'halogène ou un groupe choisi parmi un groupe alkyle en (C₁-C₄) et un groupe alkoxy en (C₁-C₄),
- R'' est un atome d'hydrogène ou un groupe alkyle en (C₁-C₄),

ou l'un de ses sels pharmaceutiquement acceptables.

8. Utilisation selon les revendications 3 à 6, dans laquelle ledit médicament candidat ou vaccin candidat est un dérivé de quinoléine de formule (II) :



dans lequel

- n est 1 ou 2 et R, indépendamment, représente un atome d'hydrogène, un atome d'halogène ou un groupe

choisi parmi un groupe alkyle en (C₁-C₃) ; un groupe -CN ; un groupe hydroxyle ; un groupe -COOR₁ ; un groupe fluoroalkyle en (C₁-C₃) ; un groupe -NO₂ ; un groupe -NR₁R₂ avec R₁ et R₂ étant un atome d'hydrogène ou un groupe alkyle en (C₁-C₃) ; et un groupe alcoxy en (C₁-C₄),

- R' est un atome d'hydrogène, un atome d'halogène ou un groupe choisi parmi un groupe alkyle en (C₁-C₄) et un groupe alcoxy en (C₁-C₄),

- R" est un atome d'hydrogène ou un groupe alkyle en (C₁-C₄),

ou l'un de ses sels pharmaceutiquement acceptables.

9. Utilisation *in vitro* ou *ex vivo* d'au moins un miARN, ledit au moins un miARN étant miR-124, en tant que biomarqueur d'une activité d'un dérivé de quinoléine ou l'un de ses sels pharmaceutiquement acceptables, sur une infection virale.

10. Utilisation selon la revendication 9, dans laquelle ledit dérivé de quinoléine est de formule (I) telle que définie dans la revendication 7, ou de formule (II) telle que définie dans la revendication 8, ou l'un de ses sels pharmaceutiquement acceptables, par exemple la 8-chloro-N-[4-(trifluorométhoxy)phényle]-quinoléin-2-amine ou la 8-chloro-N-[4-(trifluorométhyle)pyridin-2-yl]quinoléin-2-amine.

11. Utilisation selon l'une quelconque des revendications 9 ou 10, dans laquelle un taux d'expression mesuré dans un échantillon biologique isolé est comparé à une valeur de référence témoin, et dans laquelle une augmentation dudit taux mesuré par rapport à ladite valeur de référence témoin est indicative d'une activité dudit dérivé de quinoléine.

12. Procédé *in vitro* ou *ex vivo* d'évaluation d'une infection virale chez un patient présumé être infecté par un virus, comprenant au moins les étapes de :

a- mesure d'une présence ou d'un taux d'expression d'au moins un miARN, ledit au moins un miARN étant miR-124, dans un échantillon biologique précédemment obtenu à partir dudit patient ; et

b- comparaison de ladite présence ou dudit taux d'expression à une valeur de référence témoin,

dans lequel une présence ou un taux d'expression modulé dudit miARN par rapport à ladite valeur de référence témoin est indicatif d'une infection virale.

13. Procédé *in vitro* ou *ex vivo* d'évaluation d'une activité d'un dérivé de quinoléine selon l'une quelconque des revendications 9 ou 10 pour la prévention et/ou le traitement d'une infection virale chez un patient traité avec ledit dérivé de quinoléine, comprenant au moins les étapes de :

a- mesure d'une présence ou d'un taux d'expression d'au moins un miARN, ledit au moins un miARN étant miR-124, dans un premier échantillon biologique précédemment obtenu à partir dudit patient avant l'administration dudit dérivé de quinoléine et dans un deuxième échantillon biologique précédemment obtenu à partir dudit patient après l'administration dudit dérivé de quinoléine ; et

b- détermination du fait que ladite présence ou ledit taux d'expression est ou non modulé dans le deuxième échantillon biologique obtenu après le traitement par rapport au deuxième échantillon biologique obtenu avant le traitement ;

dans lequel une présence ou un taux d'expression modulé dudit miARN témoin est indicatif d'une activité dudit dérivé de quinoléine.

14. Procédé *in vitro* ou *ex vivo* de criblage d'un médicament candidat ou un vaccin candidat présumé être efficace dans la prévention et/ou le traitement d'une infection virale, comprenant au moins les étapes de :

a- traitement d'au moins une cellule isolée capable d'exprimer au moins un miARN, ledit au moins un miARN étant miR-124, avec ledit candidat, ladite cellule étant dans des conditions adaptées pour exprimer ledit au moins un miARN,

b- mesure d'une présence ou d'un taux d'expression dudit au moins un miARN,

c- comparaison de ladite présence mesurée ou dudit taux d'expression à une mesure ou un taux d'expression dudit au moins un miARN dans une cellule isolée non traitée,

dans lequel une présence ou un taux d'expression modulé dudit miARN est indicatif de l'efficacité dudit médicament candidat ou vaccin candidat sur une infection virale.

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15. Utilisation *in vitro* ou *ex vivo* d'une sonde d'acide nucléique isolée pour s'hybrider spécifiquement à miR-124, en tant qu'agent diagnostic pour mesurer une présence ou un taux d'expression de miR-124, pour diagnostiquer une infection virale pour évaluer une activité d'un médicament candidat ou d'un vaccin candidat pour prévenir et/ou traiter une infection virale.

16. Utilisation selon la revendication 15, dans laquelle ladite sonde d'acide nucléique est constituée d'une séquence d'acide nucléique choisie dans le groupe constitué de SEQ ID NO: 6 à SEQ ID NO: 87.

REFERENCES CITED IN THE DESCRIPTION

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